INVESTIGATION OF EPIDEMIC ACUTE HAEMORRHAGIC CONJUNCTIVITIS IN 1986

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Abstract

An epidemic of acute haemorrhagic conjunctivitis (AHC) started in Karachi in July, 1986 and quickly spread to other parts of the country. The epidemic died by the end of September, 1986. The usual clinical picture of acute conjunctivitis was observed, however, haemorrhages were seen in only 3.0% of the cases.
The aetiological agent was identified as a variant of the Coxsackie virus type A 24, which seems to have appeared in this part of the world for the first time (JPMA 38: 313, 1988).

INTRODUCTION

In the first week of July, 1986, reports were received of the occurrence of large number of cases of acute conjunctivitis in Karachi. The disease was reported to be highly infectious and most of the cases were among close family contacts. The disease spread to other parts of the country and was recorded in all the major cities and towns. The epidemic died by the end of September, 1986.
Clinically, the disease had a rapid onset, involving both eyes in majority of the cases. There was intense irritation, a sense of foreign body, lachrymation, photophobia, oedema of the lids with serous or seromucous discharge. In about 60% of the cases the preauricular lymph nodes were enlarged and palpable. A distinct feature was lack of frank haemorrhages and blotches and only about 3% of the cases presented with petecheal spots. The acute episode lasted for 2-3 days which cleared in 7-10 days and left no residual local or systemic complications. The paper describes the laboratory investigations undertaken to study the aetiological agent and its virological characteristics.

MATERIAL AND METHODS

Keeping in view our previous experiences and the clinical features, viral aetiology was strongly suspected., Bacteriulogical studies done did not yield any significant pathogen.

Specimen Collection and Processing.

Eye swabs were collected in virus transport media from patients with AHC attending hospitals in Rawalpindi/Islamabad and sent to the Virology Department of the National Institute of Health, Islamabad. Samples were also collected from cases occurring among the employees of the Institute.
A total of 83 specimens were collected. The initial four specimens inoculated into cell culture tubes of MRC-5, Hep 2C, LLCMK and Rhesus PMK. An enterovirus like, rapidly developing CPE, which destroyed the entire sheet within 3 days was observed in MRC-5 only. The isolate was passaged in MRC-5 and Hela cells. All other specimens were inoculated into Hela cell line as it was found to be quite sensitive in picking up the virus. Acute and convalescent phase sera were also obtained from the specimens collected ‘from the Institute.

Virological Studies

One of the isolates was subjected to detailed virological studies like Electron. microscopy, Acid stability, Chloroform sensitivity[1], Haemagglutination and Animal pathcgenicity[2]. A virus neutralization test was performed using intersecting pools of enteroviruses and EV 70. Neutralization tests in

microtitre plates performed according to the method of Yin-Murpay[3] did not give convincing results and instead, tube neutralization test was used.
The antiserum to Coxsackievirus type A 24 (EH 24) received from NIH, Japan contained 100 units of antibody and a tube neutralization test was performed using 20 units of antibody with virus dilutions at 1:100 and 1:100,000[1]. Neutralization test was also performed n 9 acute and convalescent phase sera collected from patients.

RESULTS

In all 83 samples were collected and 24 (29%) isolates were made but no agent giving a CPE similar to those caused by adeno virus or any other virus was obtained. Using the electron microscope, the cell supematant showed virus particles similar to enteroviruses (Figure 1).

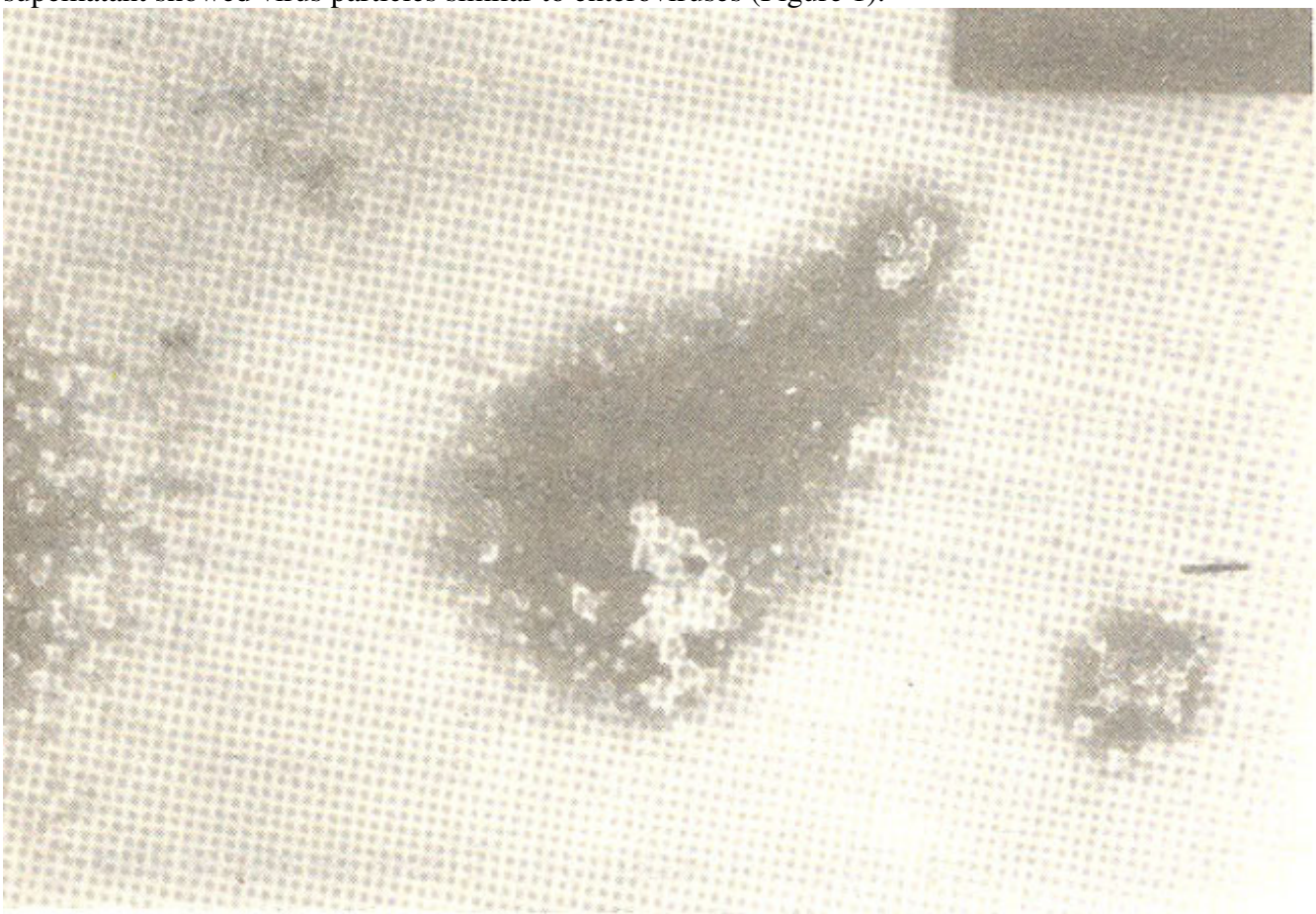

Figure 1. Electron Micrograph prepared from cell culture supernatant showing Enterovirus like particles (2x58,000).

The viral agent was found resistant to acid pH and was not inactivated by chloroform. The virus did not show haemagglutination with human group '0' cells at pH 5.8 and 7.4 and incubation at 4°C and 37 C. The animal pathogenicity test showed that mice started to show signs of illness on 9th and 10th day after inoculation with typical foot drop of forelimb and paralysis of hindlimb (Figure 2)

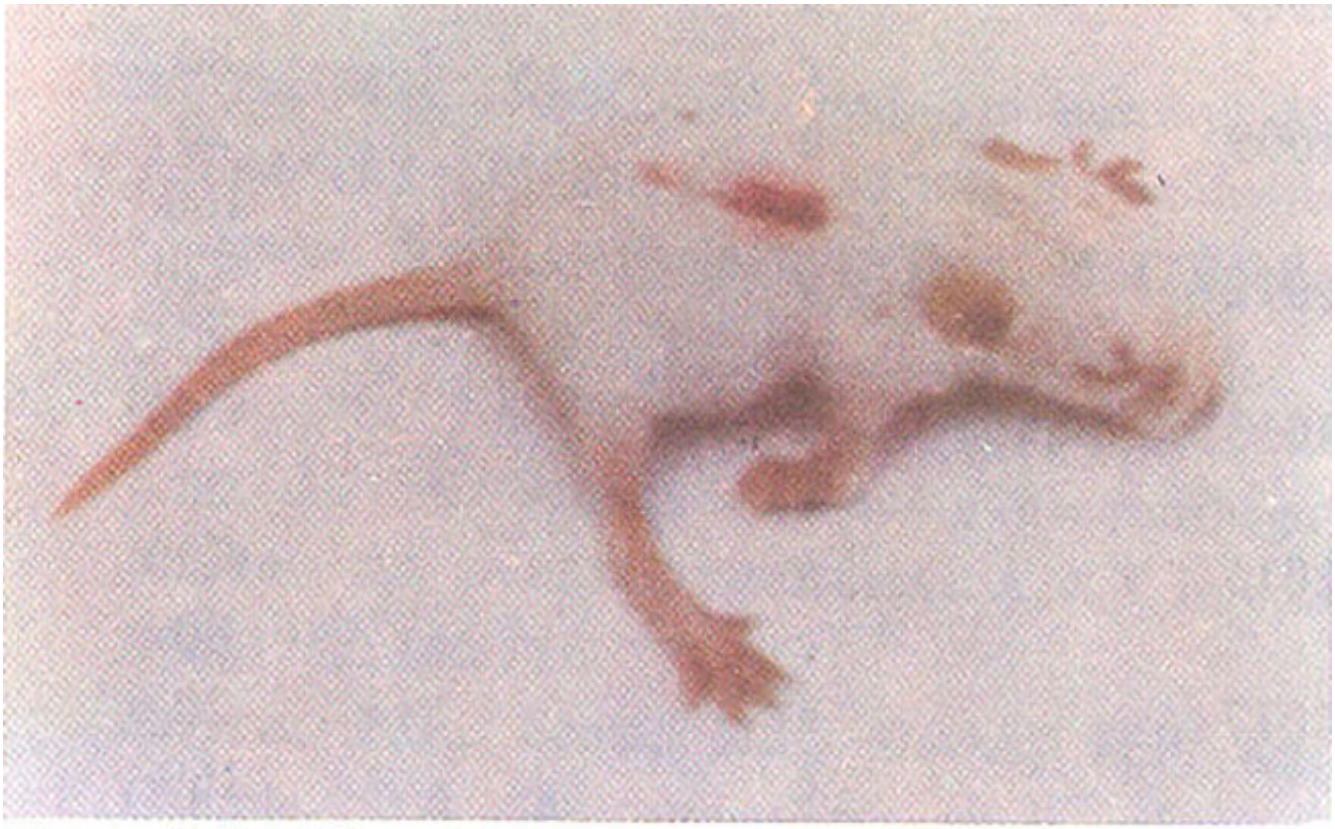

Figure 2. Suckling Mouse 9th day after inoculation by infected cell culture supernatant showing foot drop of fore limb and paralysis of hind limb.

and developed generalised paralysis before death. The histological picture (Figure 3)

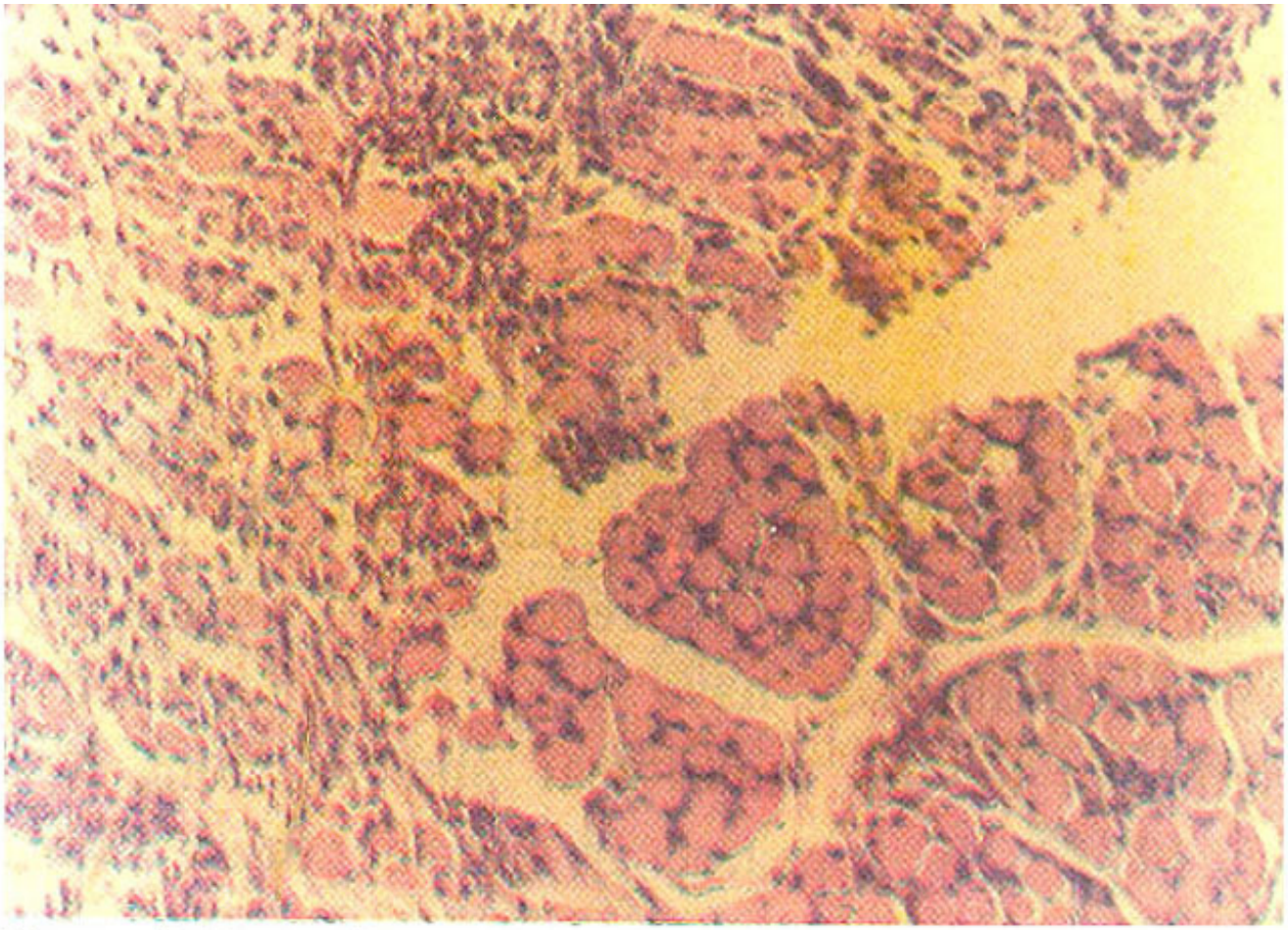

Figure 3.A section of skeletal muscle taken from the forelimb of an inoculated mouse showing marked degeneration of muscle fibres, oedema and inflammatory cellular infiltration.

showed wide spread lesions of skeletal muscles with oedema, degeneration and inflammatory cellular infIltration.

The viral agent was not neutralized by the intersecting pools of enterovirus antisera nor by EV7O antiserum. The virus gave a titre of TCID50 at log 6.5/mi. The tube neutralization with EH24 antiserum neutralized the agent thus confirming that the agent responsible for the outbreak was a variant of the Coxsackievirus type A 24 similar to the one which caused the epidemic in Singapore in l97O 4 Four virus isolates were sent to Dr. Yamazaki of the National Institute of Health, Tokyo, Japan, who also confirmed the agent to be variant of the Coxsackievirus Type 24A.

Results of the neutralizing antibody in acute and convalescent phase sera of patients are shown in Table.

TABLE. Neutralization Antibody Titres in acute and convalescent Phase Sera.

Sample No.	Serum	Neutralization Antibody Titre to CA 24.
1003	a	⩽20
	c	80
1009	a	20
	c	160
1012	a	⩽20
	c	80
1013	a	⩽20
	c	20
1015	a	⩽20
	c	80
1017	a	20
	c	160
1021	a	⩽20
	c	40
1029	a	⩽20
	c	20
1030	a	⩽20
	c	40

a – acute phase
c – convalescent phase

There was a fourfold rise in antibody level in 5 cases out of 9.

DISCUSSION

It has been reported that CA 24 variant has been responsible for epidemics of AHC in South East Asia and was first recorded in Singapore in 1974. Outbreaks have also been recorded in Malaysia in the same year and in Hong Kong in l971[5] It appeared in mixed outbreak with EV7O in Southern India in 1975 followed by epidemics in coastal areas of India and South East Asia[6]. It again appeared in Malaysia, Sri Lanka and Southern India in 1978. Another reported epidemic was in Vellore, Southern India in: 1980. Prior to 1986, CA 24 had not been reported outside South East Asia and the Indian subcontinent[7]. In 1986, CA24 was isolated during outbreaks in Taiwan, American Samoa, and India. (CDC unpublished data) [8]. It is possible that CA24, like EV7O, may spread to other areas in the western hemisphere. The occurrence of AHC due to CA24 variant in Pakistan, is of special significance. The disease started in the city of Karachi during the monsoon season when heat and humidity were favourable for finger-fomite.eye spread. Karachi has many high density population areas. There is speculation that the disease might have been brought to Karachi by visitors from South India. The disease did not remain localised in Karachi but spread to other parts of the country and was recorded as far north as Peshawar, more than 1500 km away from Karachi, and within 3 months, the epidemic swept through the entire country. This is the first time that the A.H.C. due to CA24 variant has been recorded in the country.

A significant clinical feature of the disease was that it did not cause haemorrhage as frequently as were caused by EV7O in 1981. In the latter epidemic, the incidence of conjunctival haemorrhage was 50% while in the present epidemic, very few patients (3.0%) showed petecheal spots and no blotches or frank haemorrhages were observed. Bahrin, Joshi and Yin-Murphy[9] while investigating the outbreak of acute conjunctivitis in Brunei in 1975, reported haemorrhages in 1% of the cases and in 11% during the epidemic of 1970 in Singapore[4]. Chirstopher et al[10] reported haemorrhages in 8% of the cases while investigating an epidemic of A.H.C. in 1979 in Veilore, India.

Laboratory investigation of the disease is easy and the virus can be detected using the Hela and MRC-5 cell lines. The virus could not be isolated in Hep 2C, or Rhesus PMK cells but grew rapidly in Hela and MRC-5 cell lines in corn. parison to EV7O. Isolations were made at 33°C and 37°C. The technique given by Yin-Murphy[3] did not give convincing results. Tube method for neutralization test gave better results. The agent has been found to be pathogenic for suckling mice in which it caused exterjsve degeneration of skeletal muscles, with typical forelimb drop and flaccid deformities. Higgins et al [11] compared different strains from various outbreaks and observed that Singapore 1970 and HK 3751/71 strains were pathogenic in suckling mice. Strain HK 3751/71 was more pathogenic and caused greater morbidity and mortality among mice even on primary inoculation. Virus strains isolated by Christopher et a1[10] were pathogenic to suckling mice ‘after third or fourth passage. The strains isolated in Pakistan were pathogenic to animals on first isolation and primary inoculation. This may represent strain variation and detailed studies of various strains might help to resolve the virological characteristics and its epidemiological features.

Seroconversion studies were very limited as only 9 paired sera could be obtained. A fourfold rise in antibody titre was obtained in only 5 cases and the highest titre observed was 1:160.

However, it has also been observed by other workers[4-10] that antibody response to Coxsackievirus A 24 in patients with conjunctivitis is generally low.

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